Cross-sex hormone treatment in male-to-female transsexual persons reduces serum brain-derived neurotrophic factor (BDNF)

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Abstract
Serum levels of brain-derived neurotrophic factor (BDNF) are reduced in male-to-female transsexual persons (MtF) compared to male controls. It was hypothesized before that this might reflect either an involvement of BDNF in a biomechanism of transsexualism or to be the result of persistent social stress due to the condition. Here, we demonstrate that 12 month of cross-sex hormone treatment reduces serum BDNF levels in male-to-female transsexual persons independent of anthropometric measures. Participants were acquired through the European Network for the Investigation of Gender Incongruence (ENIGI). Reduced serum BDNF in MtF thus seems to be a result of hormonal treatment rather than a consequence or risk factor of transsexualism.

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1. Introduction
Transsexualism is a condition characterized by a strong and persistent desire to belong to the other sex. An etiological reason for transsexualism has not been identified yet, but psychological and biological factors have been discussed that might influence gender identity (Schneider et al., 2006;
Auer et al., 2013a). From a neurobiological perspective, there is evidence for altered brain structure volumes in transsexual persons determined by magnetic resonance imaging (Zubiauen-Elloza et al., 2014; Savic and Arver, 2011). It is, however, not clear if these brain alterations are correlates of transsexualism or merely a result of cross-sex hormone treatment, chronic social stress or long-term gender dysphoria (Zubiauen-Elloza et al., 2014). Moreover, serum levels of the neurotrophin BDNF were reported to be lower in MtF in comparison to cisgender men (Fontanari et al., 2013). BDNF is a neurotrophic factor involved in a variety of central processes, including neurogenesis, neuronal maturation and synaptogenesis. As a factor that influences brain plasticity, serum BDNF also impacts on macroscopic brain morphology (Rizos et al., 2011) and thus is one of the factors that may be involved in the abovementioned neuroimaging findings. In MtF transsexual persons cross-sex hormone treatment decreases brain volume (Pol et al., 2006), yet the influence of cross-sex hormone treatment on serum BDNF has not been studied so far. Lower BDNF-levels in MtF persons were postulated to indicate early and persistent distress in MtF, potentially even influencing a neurobiological basis for transsexualism (Fontanari et al., 2013) or a biomarker for social vulnerability in individuals diagnosed with gender dysphoria. However, given the evidence that BDNF-levels are sex-dependent, sex-steroids may also have a role in this context (Fuss et al., 2013). Thus the present study was designed to evaluate the influence of cross-sex hormone treatment on serum BDNF levels. We measured serum BDNF in MtF-transsexual persons before and after one year of cross-sex hormone treatment.

2. Experimental procedures

The subjects investigated in this study (n=20) were part of the European Network for the Investigation of Gender Incongruence (EnIGI), a collaboration of four European gender identity clinics (Amsterdam, Ghent, Hamburg, and Oslo) to study the diagnostics and treatment of transsexualism (Kreukels et al., 2012).

The patients included in the present study had been exclusively diagnosed and treated at the Department of Endocrinology at the Ghent University Hospital between February 2010 and August 2012. All patients were from Caucasian origin and hormone-naïve at first visit. The choice for mode of hormone treatment depended on the age of the corresponding subject, according to the increased cardiovascular risk profile in older MtF.

MtF below the age of 45 years (N=13) were treated with 50 mg of Cyproteronacet (CA) (Androcur®, Bayer) in addition to 4 mg of estradiol valerate (EV) (Progynova®, Bayer) daily, while those being older than 45 years received 50 mg of CA daily and a transdermal 17β-estradiol (E2) patch releasing 100 μg/24 h (Dermestril®, Bessins, Belgium) (N=7). According to their own choice and in agreement with the treating mental health professional, some patients (N=9) received a sequential hormone protocol, starting with the antiandrogen alone for about 3 months and estradiol was then added later on. One patient was excluded from further analysis due to inappropriate high testosterone as well as LH and FSH levels (>2 SD) at 12 month follow-up, indicating low treatment adherence.

This study was approved by the ethical review board of the Ghent University Hospital. The study was conducted in accordance with the Declaration of Helsinki and all participants gave written informed consent. This study is registered at clinicaltrials.gov. Clinical trial number: NCT01072825.

2.1. Medical history and examination

Data on comorbidities, life-style including smoking history, alcohol intake, medical history and medication use where acquired from each patient by means of questionnaires and corresponding information was compared to the clinical information from the medical chart files. Physical activity was measured using the Baecke habitual physical activity questionnaire (Baecke et al., 1982).

2.2. Anthropometrics and laboratory measures

A detailed description of the assessments of anthropometry and laboratory evaluation is described elsewhere (Wierckx et al., 2014). The presented patients had been part of a larger cohort of patients and their anthropometric changes had been reported in an earlier study with a focus on safety issues and effectiveness of cross-sex hormone treatment (Wierckx et al., 2014). Briefly, height was measured to the nearest 0.1 cm using a Harpenden stadiometer (HoltainLtd, Crymch, UK). Body weight was measured in light indoor clothing without shoes to the nearest 0.5 kg. Body composition was measured using dual-energy X ray absorptiometry with a Hologic Discovery machine (Hologic Inc., Bedford, MA, USA). Blood drawings took place in the morning between 8:00 and 9:00 a.m. following an overnight fast. After a clotting period of 30-60 min, serum was centrifuged and stored at –80 °C until analysis. 17β-Estradiol (E2), testosterone and cortisol were determined using liquid chromatography tandem mass spectrometry (AB Scieix 5500 triplequadrupole mass spectrometer; AB Scieix, Toronto, Canada). For information on the methods of the determination of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and sex hormone binding globulin (SHBG) and interassay coefficients of variance (CVs) please see (Wierckx et al., 2014).

2.3. Measurement of BDNF levels

Endogenous levels of BDNF were measured in the rethawed serum samples using improved ELISA kits according to the manufacturer’s instructions (Promega Inc., Mannheim, Germany) adapted to the fluorometric technique also used for nerve growth factor determination as described (Helliweg et al., 2006). A previous study using this BDNF assay procedure showed a detection limit of 0.7 pg/ml serum BDNF (Helliweg et al., 2008) and coefficients of inter- and intra-assay variation of 8.7±5.2% and 13.2±1.6%, respectively (Helliweg et al., 2008).

2.4. Mental health assessment

General well-being and mental health was assessed by means of non-standardized questionnaires on present symptoms, covering the dimensions anxiety (“Feeling anxious”), panic attacks (“Do you suffer from panic attacks”), emotionality (”I am nervous or quickly irritated”, “I am crying faster and feel emotionally unstable”, “I suffer from mood swings”) all scaled as follows: 0=no 1=mild 2=moderate 3=severe. At baseline all participants further underwent a Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I).

2.5. Statistical analysis

Statistical analysis was carried out using PASW 18.0 (SPSS Inc., Chicago, IL). Data are reported as means (±S.E.M.). For longitudinal comparison a paired t-test was performed for metric variables and the Wilcoxon Signed Ranks Test for ordinal variables. Correlations with BDNF were examined using Pearson’s correlation coefficient. Significance was evaluated at a probability of 5% or less (<0.05).
Cross-sex hormone treatment had no influence on body weight or body mass index (BMI) but significantly altered body-composition by increasing total fat mass and reducing total lean mass (p < 0.001).

As expected, induction of cross-sex hormone treatment resulted in a female hormonal milieu with a decrease in testosterone and an increase in estradiol levels. There was no significant effect on morning cortisol levels. During the 12 month of follow-up there was no significant change in any measure of physical activity levels. With respect to the non-standardized variables on mood and general well-being a significant change in the tendency to cry (p = 0.002) was reported. Two patients had a positive history for major depression though they were not currently suffering from depressed mood at baseline evaluation. These two patients were taking antidepressant medication at baseline. None of the patients had been newly hospitalized for any psychiatric disorder during the 12 month follow-up period. Paired t-test revealed significantly lower serum BDNF after 12 month of cross-sex hormone treatment in MtF (p = 0.014; Fig. 1). The effect persisted after excluding two subjects who had been on antidepressant drugs at baseline evaluation (p = 0.006). Serum BDNF before and after 12 month of treatment were highly correlated (R = 0.81, p < 0.001). In contrast, we found no correlation between serum BDNF and age, weight, BMI, total fat mass, total lean mass, LH, FSH, estradiol, testosterone, cortisol, physical activity or pack years (for more details of these variables see Table 1) neither before nor after 12 month of cross-sex hormone treatment.

4. Discussion

In the present study, we found that after 12 month of cross-sex hormone treatment serum BDNF in MtF persons is reduced. Since cross-sex hormone treatment in MtF is accompanied by bodily changes, we correlated serum BDNF with anthropometric measures before and after cross-sex hormone treatment and found no relation. Thus, our data suggest that cross-sex hormone treatment reduces serum BDNF independent of bodily changes. So far, interactions of sex steroids and BDNF have been extensively studied in rodents, but data in human subjects are rare. Women in general seem to have lower BDNF levels than men (Lomnatsch et al., 2005). In women with amenorrhea or menopause BDNF levels are lower than in fertile women, and menopause status may predict BDNF levels (Begliuomini et al., 2007). Interesting, hormone replacement therapy is capable of restoring BDNF to the level of fertile women (Begliuomini et al., 2007) suggesting a direct relation between hormones and BDNF levels. Furthermore, BDNF levels tend to fluctuate during the menstrual cycle (Lomnatsch et al., 2005). Yet, it is not clear if this is due to a direct regulation of BDNF through sex steroids, or if BDNF as well as sex steroids are independently regulated during the menstrual cycle (Begliuomini et al., 2007) as both BDNF and sex steroids can originate from the ovaries (Pluchino et al., 2009). The present study indicates that sex steroids can directly alter serum BDNF. Moreover, BDNF is expressed in a variety of tissues and BDNF levels in serum are regarded to be closely related to BDNF concentrations in the brain (Karege et al., 2002) and may provide information on BDNF release and uptake by the central nervous system (Rasmussen et al., 2009). This may also explain for the observed decrease in brain volume following androgen deprivation therapy in MtF (Pol et al., 2006) as well as in patients with advanced prostate cancer (Chao et al., 2013). In further studies, particular attention should therefore be paid to the interaction effects of changes in the hormonal milieu with decreased BDNF levels and brain volume.

In addition to the potential effect of estradiol, the decrease in BDNF may also be attributable to testosterone withdrawal, as part of cross-sex hormone therapy consists in the co-administration of the anti-androgenetic progestin cyproterone acetate (CA). However, only few studies have evaluated the direct influence of testosterone on BDNF-levels and in particular studies in humans are lacking. In rodents, testosterone depletion by means of gonadectomy has been shown to decrease BDNF levels in motoneuron populations (Verhovshek et al., 2010) as well as in the hippocampus (Li et al., 2012), an effect that is reversible by subsequent testosterone substitution.

Earlier it had been hypothesized that lower BDNF in MtF compared to healthy male controls could be related to the “psychological abuse” i.e. stigma, prejudice, and discrimination in the MtF population (Fontanari et al., 2013) and thus basically reflect disturbed mental health. This hypothesis stemmed from a large body of literature claiming a relation between BDNF and mood disorders or schizophrenia. For example a recent meta-analysis by Molendijk et al. (2014) found that altered serum BDNF concentrations can be a peripheral correlate of depression. Serum BDNF is also influenced by other factors, which are not directly related to mood like smoking status, age, BMI, physical exercise and hormonal status (Bus et al., 2011). A major limitation of the present study is that we had no data on mental health status at 12 month follow-up, assessed by means of standardized questionnaires or clinical interviews in the ENIGI study. The information on mental health that we had indicated no major mood disturbances in our sample. More importantly, it has been repeatedly reported that initiation of hormonal treatment is accompanied with improvements in mood.
(Colizzi et al., 2014; Auer et al., 2013b) and 78% of MtF persons report a significant improvement of psychiatric symptoms following transition (Murad et al., 2010). Therefore, we would rather expect an elevation of BDNF level if it would mainly be determined by psychiatric symptoms in the studied cohort. Social stigma and isolation may however persist after transition and were not measured in the present study, which is another limitation.

To sum up, previous studies suggested that in females estradiol levels are positively related to serum BDNF while testosterone withdrawal is associated with lower BDNF-levels. Therefore our data are in agreement with the literature and demonstrate that the introduction of a female hormonal status in biological men reduces serum BDNF. Further studies should investigate if change in BDNF levels is primarily driven by testosterone withdrawal or estradiol substitution. Moreover, the sequelae of chronically reduced BDNF should be studied in relation to emotion and cognition, although it was demonstrated before that cross-sex hormone treatment does not alter cognitive performance (Miles et al., 2006).

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